L7: Entry 10 of 34 File: USPT Sep 9, 2003

DOCUMENT-IDENTIFIER: US 6617128 B2
TITLE: Nucleic acid molecules encoding proteins which impart the adhesion of neisseria cells to human cells

Brief Summary Text (3):
To the genus Neisseria (gram-negative cocci) belong a number of bacterial species which, being saprophytes, populate the upper human respiratory tract. Apart from commensal species (e.g.: N. sicca) and opportunistically pathogenic species (e.g.: N. lactamica), two Neisseria species are known which clearly possess human-pathogenic properties. One of the species is N. gonorrhoeae, the pathogen of the venereal disease gonorrhea, which exclusively occurs in humans, and N. meningitidis, the pathogen of the bacterial epidemic meningitis. In both cases the etiology, that is the causal connection between the development of the clinical picture and the population by bacteria from said species has meanwhile been substantiated.

Brief Summary Text (4):
The purulent meningitis (Meningitidis cerebrospinalis epidemica) caused by N. meningitidis ("meningococcus"), which usually is epidemical, is a systemic invasive infection of the human meninx and spinal meninx. Occasionally, hemorrhagic exanthema at the trunk or concomitant diseases caused by Herpes simplex can be observed in addition. The pathogen can appear in the form of several serotypes, which are distinguishable by means of agglutination assays with immune sera. The main groups differ remarkably, and their prevalence differs with regard to when and where they appear. Meningococcus meningitidis has up to now occurred in large numbers every 8 to 12 years with the increased prevalence lasting between 4 to 6 years. While serovar B meningococci brought on 50% to 55% of the recent diseases to the civilian population as well as to the military personnel in the United States, most epidemic diseases in the United States during the first half of the century were caused by serovar A meningococci.

Brief Summary Text (6):
Usually, the diseases caused by N. gonorrhoeae and N. meningitidis are treated with antibiotics. More and more, however, the bacteria are becoming resistant to single or groups of the antibiotics used so that the therapy method that has nearly exclusively been used up to now will most likely not be successful in the long run. Therefore, it is desirable and urgent that alternative therapy methods, preferably preventive ones, be developed.

Brief Summary Text (7):
Neisseria gonorrhoeae and N. meningitidis exclusively occur in humans. They have adapted to the host organism and show a number of properties that are able to make the defense mechanisms of the host ineffective. Therefore, up to now there is no vaccine available that prevents gonorrhea. This is to a limited extent also true for meningococcus meningitidis. Even though the disease has recently been caused mainly by bacteria of the same serovar, group B, no effective vaccine against meningococci of group B has existed up to now. Vaccines against other serovars only offer partial protection and are not unproblematic from an immunological point of view. The reason for the failure of the immune defense is, inter alia, the antigen

variation of the pathogens, which in the case of the pathogenic Neisseria is particularly developed. However, a limitation of the free development of the antigen variation seems to be necessary where the functional region has to be sterically maintained in order to guarantee the interaction with conserved and constant structures of the host receptors. This requirement especially applies to the adhesins that serve for adhering to the host cell. Only if the functional region that is involved in the physical interaction is kept constant, the interaction with the receptor of the host cell is possible. This region should be excluded from antigen variation to a large extent and is therefore a suitable starting-point for the development of a new therapy method.

Brief Summary Text (18):
In a preferred embodiment the nucleic acid molecule of the invention originates from a pathogenic Neisseria species, in particular from Neisseria gonorrhoea or Neisseria meningitidis.

CLAIMS:

1. An isolated nucleic acid molecule selected from the group consisting of (a) a nucleic acid molecule comprising the nucleotide sequence depicted in SEQ ID NO: 1; (b) a nucleic acid molecule comprising a nucleotide sequence having 95% sequence identity to SEQ ID NO:1 due to the degeneracy of the genetic code; and (c) a nucleic acid molecule comprising a nucleotide sequence that hybridizes under stringent hybridization conditions of 0.2.times.SSC, 0.1% SDS and 68.degree. C. to the complement of SEQ ID NO: 1; wherein an open reading frame contained in the nucleic acid molecule encodes a protein from bacteria of the genus Neisseria that mediates the adhesion of Neisseria cells to human cells.

2. The isolated nucleic acid molecule according to claim 1, wherein the molecule originates from a pathogenic Neisseria species.

3. The isolated nucleic acid molecule according to claim 2, wherein the Neisseria species is Neisseria gonorrhoeae or Neisseria meningitidis.

4. A vector comprising a nucleic acid molecule according to claim 1.

5. A host cell comprising the nucleic acid molecule according to claim 1.

6. An isolated fragment of the nucleic acid molecule according to claim 1 encoding a lipoprotein or biologically active fragment of said lipoprotein that mediates adhesion of Neisseria cells to human cells from a bacteria of the genus Neisseria selected from the group consisting of (a) a nucleic acid molecule encoding a protein having the amino acid sequence as depicted in SEQ ID NO:7; (b) a nucleic acid molecule encoding a protein having the amino acid sequence depicted in SEQ ID NO:7 from amino acid residue 19 to amino acid residue 320; (c) a nucleic acid molecule comprising a nucleotide sequence having 95% sequence identity to (i) a nucleotide sequence encoding a protein comprising SEQ ID NO:7, and (ii) a nucleotide sequence encoding a protein having the amino acid sequence depicted in SEQ ID NO:7 from amino acid residue 19 to amino acid residue 320; and (d) a nucleic acid molecule comprising a nucleotide sequence that hybridizes under stringent hybridization conditions of 0.2.times.SSC, 0.1% SDS and 68.degree. C. to (i) the complement of a nucleotide sequence encoding a protein comprising SEQ ID NO:7, (ii) the complement of a nucleotide sequence encoding a protein having the amino acid sequence depicted in SEQ ID NO:7 from amino acid residue 19 to amino acid residue 320.

7. The nucleic acid molecule according to claim 6, wherein the lipoprotein or biologically active fragment of said lipoprotein has the ability to adhere to human cells.

8. The nucleic acid molecule according to claim 7, wherein the protein or biologically active fragment possesses

the ability to adhere to human cells in complexes with the protien PilC.

9. A vector comprising a nucleic acid molecule according to claim 6.

10. The vector according to claim 9, wherein the nucleic acid molecule is operatively linked to at least one regulatory DNA element allowing the expression of said nucleic acid in a prokaryotic or a eukaryotic cell.

11. A host cell comprising a nucleic acid molecule according to claim 6.

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L2: Entry 25 of 29 File: USPT Nov 2, 1999

DOCUMENT-IDENTIFIER: US 5976536 A
**** See image for Certificate of Correction ****
TITLE: Neisseria mutants, lipooligosaccharides and immunogenic compositions

Brief Summary Text (4):
Neisseria meningitidis and Neisseria gonorrhoeae are important human pathogens. N. meningitidis causes meningitis, sepsis and bacteremia; N. gonorrhoeae causes gonorrhoea in both sexes, pelvic inflammatory disease and/or sterility in women, and rectal and pharyngeal infections, as in homosexual men. More rarely, disseminated gonococcal infection (gonococcal bacteremia) can result, with complications such as polyarthralgias or purulent arthritis, for example. These two species are relatively closely related genetically; there is approximately 85% DNA sequence homology between the genomes of the two species. The genus also includes several other species which are nonpathogenic to man although they colonize the upper respiratory tract.

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L2: Entry 23 of 29 File: USPT Sep 11, 2001

DOCUMENT-IDENTIFIER: US 6287574 B1
TITLE: Proteinase K resistant surface protein of neisseria meningitidis

Detailed Description Text (129):
A LambdaGEM-11 genomic DNA library from Neisseria meningitidis strain 608B (B:2a:P1.2) was constructed according to the manufacturer's recommendations (Promega CO, Madison, Wis.). Briefly, the genomic DNA of the 608B strain was partially digested with Sau 3AI, and fragments ranging between 9 and 23 Kb were purified on agarose gel before being ligated to the Bam HI sites of the LambdaGEM-11 arms. The resulting recombinant phages were used to infect Escherichia coli strain LE392 (Promega) which was then plated onto LB agar plates. Nineteen positive plaques were identified after the immuno-screening of the library with the Neisseria meningitidis 22 kDa surface protein-specific monoclonal antibodies of Example 2 using the following protocol. The plates were incubated 15 minutes at -20.degree. C. to harden the top agar. Nitrocellulose filters were gently applied onto the surface of the plates for 30 minutes at 4.degree. C. to absorb the proteins produced by the recombinant viral clones. The filters were then washed in PBS-Tween 0.02% (vol/vol) and immunoblotted as described previously [Lussier et al., J. Immunoassay, 10, p. 373 (1989)]. After amplification and DNA purification, one viral clone, designated clone 8, which had a 13 Kb insert was selected for the subcloning experiments. After digestion of this clone with Sac I, two fragments of 5 and 8 Kb were obtained. These fragments were purified on agarose gel and ligated into the Sac I restriction site of the low copy number plasmid pWKS30 [Wang and Kushner, Gene, 100, p. 195 (1991)]. The recombinant plasmids were used to transform Escherichia coli strain JM109 (Promega) by electroporation (Bio-Rad, Mississauga, Ont., Canada) following the manufacturer's recommendations, and the resulting colonies were screened with the Neisseria meningitidis 22 kDa surface protein-specific monoclonal antibodies of Example 2. Positive colonies were observed only when the bacteria were transformed with the plasmid carrying the 8 Kb insert. Western blot analysis (the methodology was described in Example 2) of the positive clones showed that the protein expressed by Escherichia coli was complete and migrated on SDS-PAGE gel like the Neisseria meningitidis 22 kDa surface protein. To further reduce the size of the insert, a clone containing the 8 Kb fragment was digested with Cla I and a 2.75 Kb fragment was then ligated into the Cla I site of the pWKS30 plasmid. Western blot analysis of the resulting clones clearly indicated once again that the protein expressed by Escherichia coli was complete and migrated on SDS-PAGE gel like the native Neisseria meningitidis 22 kDa surface protein.

Detailed Description Text (197):
A 540 nucleotide fragment was amplified by PCR from the Neisseria meningitidis strain 608B genomic DNA using the following two oligonucleotide primers (SEQ ID NOS 27 & 28, respectively) (OCRR8: 5'-TAATAGATCTATGAAAAAAGCACTTGCCAC-3' and OCRR9: 3'-CACGCGCAGTTTAAGACTTCTAGATTA-5'). These primers correspond to the nucleotide sequences found at both ends of the 22 kDa gene. To simplify the cloning of the PCR product, a Bgl II (AGATCT) restriction site was incorporated into the nucleotide sequence

of these primers. The PCR product was purified on agarose gel before being digested with Bgl II. This Bgl II fragment of approximately 525 base pairs was then inserted into the Bgl II and Bam HI sites of the plasmid p629. The plasmid containing the PCR product insert named pNP2204 was used to transform E. coli strain DH5.alpha.F'IQ. A partial map of the plasmid pNP2204 is presented in FIG. 16. The resulting colonies were screened with Neisseria meningitidis 22 kDa surface-protein specific monoclonal antibodies described in Example 2. Western blot analysis of the resulting clones clearly indicated that the protein synthesized by E. coli was complete and migrated on SDS-PAGE gel like the native Neisseria meningitidis 22 kDa surface protein. Plasmid DNA was purified from the selected clone and then sequenced. The nucleotide sequence of the insert present in the plasmid perfectly matched the nucleotide sequence of the gene coding for the Neisseria meningitidis 22 kDa protein presented in FIG. 1.

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L2: Entry 23 of 29 File: USPT Sep 11, 2001

US-PAT-NO: 6287574
DOCUMENT-IDENTIFIER: US 6287574 B1

TITLE: Proteinase K resistant surface protein of neisseria meningitidis

DATE-ISSUED: September 11, 2001

INVENTOR-INFORMATION:

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US-CL-CURRENT: 424/250.1; 424/184.1, 424/185.1, 424/190.1, 424/249.1, 530/300, 530/350, 536/23.7

CLAIMS:

We claim:

1. An isolated polypeptide comprising a sequence as set forth in SEQ ID NO:2.

2. An isolated polypeptide comprising a sequence as set forth in SEQ ID NO:4.

3. An isolated polypeptide comprising a sequence as set forth in SEQ ID NO:6.

4. An isolated polypeptide comprising a sequence as set forth in SEQ ID NO:8.

5. An isolated polypeptide comprising a sequence selected from the group of sequences consisting of SEQ ID NO:9; SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, and SEQ ID NO:26.

6. An isolated polynucleotide encoding a polypeptide comprising a sequence as set forth in SEQ ID NO:2.

7. An isolated polynucleotide encoding a polypeptide comprising a sequence as set forth in SEQ ID NO:4.

8. An isolated polynucleotide encoding a polypeptide comprising a sequence as set forth in SEQ ID NO:6.

9. An isolated polynucleotide encoding a polypeptide comprising a sequence as set

forth in SEQ ID NO:8.

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L7: Entry 12 of 34 File: USPT Apr 1, 2003

DOCUMENT-IDENTIFIER: US 6541201 B1

**** See image for Certificate of Correction ****

TITLE: Hybridization assay probes and methods for detecting the presence of neisseria meningitiidis subtypes A, C and L in a sample

Abstract Text (1):
The present invention discloses hybridization assay probes, amplification primers, nucleic acid compositions and methods useful for detecting Neisseria nucleic acids. Hybridization assay probes and amplification primers that selectively detect Neisseria meningitidis and distinguish those Neisseria meningitidis from Neisseria gonorrohoeae are disclosed. Other hybridization probes selectively detect Neisseria gonorrohoeae and not Neisseria meningitidis are also described.

Brief Summary Text (2):
The inventions described and claimed herein relate to the design and use of amplification oligonucleotides and nucleic acid probes to Neisseria gonorrhoeae and Neisseria meningitidis which allow detection of these organisms in test samples.

Brief Summary Text (4):
The genus Neisseria includes two gram-negative species of pyogenic cocci that are pathogenic for man, and that have no other known reservoir: the meningococcus (Neisseria meningitidis) and the gonococcus (Neisseria gonorrhoeae). A number of non-pathogenic species also inhabit the upper respiratory tract of humans and may be easily confused with meningococci. Meningococcal meningitis was recognized as a contagious disease early in the 19th century and is especially prevalent among military personnel. The causative agent of meningococcal meningitis is Neisseria meningitidis.

Brief Summary Text (8):
Oligonucleotides for the amplification of nucleic acid for detection of Neisseria have been described. Biken-meyer and Armstrong, J. Clin. Microbiol. 30:3089-3094 (1992), describe probe sets for use in the ligase chain reaction directed to the Opa and pilin genes of Neisseria gonorrhoeae. Kristiansen et al. Lancet 340:1432-1434 (1992) describe primers directed to an insertion element referred to as IS1106 for amplification and detection of Neisseria meningitidis. McLaughlin et al., Mol. and Cell Probes 7:7-17 (1993) describe primers for use in the polymerase chain reaction directed to the 16S-23S rRNA internal transcribed spacer and a set of primers directed to a subregion of the 16S rRNA of Neisseria meningitidis. Probes for the detection of rRNA or rDNA sequences of Neisseria gonnorhoeae and/or Neisseria meningitidis have been described by Granato and Franz J. Clin. Microbiol. 28:944-948, (1990), Wolff, U.S. Pat. No. 5,173,401 (Dec. 22, 1992), Rossau and Van Heuverswijn, European Patent Application Publication No. 0 337 896, Hogan et al. PCT/US87/-03009, and Barns et al., U.S. Pat. No. 5,217,862 (Jun. 8, 1993).

Brief Summary Text (11):

The amplification oligonucleotides and oligonucleotide hybridization assay probes function by hybridizing to target Neisseria 16S and 23S rRNA and/or rDNA gene sequences under stringent hybridization assay conditions. In preferred embodiments, the probes and amplification oligonucleotides described herein, when used together, can distinguish Neisseria meningitidis from other microorganisms found in clinical samples such as blood or tissues and from Neisseria gonorrhoeae species. Accordingly, the amplification oligonucleotides and hybridization assay probes may be used in an assay to specifically detect and/or amplify Neisseria meningitidis-derived nucleic acids. In preferred embodiments, the hybridization assay probes described herein are able to selectively hybridize to nucleic acids from Neisseria meningitidis over those from Neisseria gonorrhoeae under stringent hybridization conditions. In some embodiments of the present invention, the hybridization assay probe comprises an oligonucleotide that contains a reporter group such as an acridinium ester or a radioisotope to help identify hybridization of the probe to its target sequence. In some embodiments of the present invention, the amplification oligonucleotide optionally has a nucleic acid sequence recognized by an RNA polymerase or which enhances transcription initiation by an RNA polymerase.

Brief Summary Text (13):
The present invention features hybridization assay probes useful for detecting nucleic acids from Neisseria meningitidis. These hybridization assay probes are preferably selected from the following nucleotide sequences: SEQ ID NO: 11 GGCTGTTGCT AATATCAGCG SEQ ID NO: 12 GGCTGTTGCT AATACCAGCG SEQ ID NO: 15 CGCTGATATT AGCAACAGCC SEQ ID NO: 16 CGCTGGTATT AGCAACAGCC SEQ ID NO: 25 GGCUGUUGCU AAUAUCAGCG SEQ ID NO: 26 GGCUGUUGCU AAUACCAGCG SEQ ID NO: 27 CGCUGAUAUU AGCAACAGCC, and SEQ ID NO: 28 CGCUGGUAUU AGCAACAGCC.

Brief Summary Text (16):
Another aspect of the present invention includes compositions for detecting Neisseria meningitidis and Neisseria gonorrhoeae that are nucleic acid hybrids formed between an oligonucleotide of the present invention and a specific region of a nucleotide polymer from a Neisseria meningitidis or Neisseria gonorrhoeae. Generally, the nucleotide polymer contains a nucleic acid sequence that substantially corresponds to an oligonucleotide sequence of the present invention or its complement and is derived from the rRNA or the rDNA encoding the ribosomal RNA of the Neisseria meningitidis or Neisseria gonorrhoeae. The oligonucleotide present in these compositions may be an amplification oligonucleotide, a helper oligonucleotide, a hybridization assay probe, or a combination thereof. Thus, compositions of the present invention may contain one or more amplification oligonucleotides, one or more helper oligonucleotides, and one or more hybridization assay probes.

Brief Summary Text (18):
The present invention also contemplates methods for detecting the presence of Neisseria in which a test sample is contacted with a nucleic acid hybridization assay probe under stringent hybridization assay conditions wherein the nucleic acid hybridization assay probe is capable of hybridizing to Neisseria meningitidis target nucleic acid sequences and not to the nucleic acid sequences from Neisseria gonorrhoeae. The present invention also contemplates oligonucleotides and the equivalents thereof used in these methods that optionally contain a reporter molecule that aids in the identification of the hybridization of the probe to its target sequence. This invention is useful for detecting the presence of Neisseria nucleic acids in test samples from humans such as blood, blood derived samples, tissues, tissue derived samples, other body fluids and body samples.

Brief Summary Text (19):
The present invention also contemplates methods for detecting the presence of Neisseria meningitidis in which the nucleic acid is amplified using at least one amplification oligonucleotide of the present invention. In preferred embodiments, that amplification is then followed by a detection step in which the amplified nucleic acid is detected using an oligonucleotide hybridization assay probe of the present invention. The methods of

the present invention also contemplate the use of amplification oligonucleotides which include the nucleotide sequence for an RNA promoter.

Brief Summary Text (23):
Another aspect of the present invention includes kits that contain one or more of the oligonucleotides of the present invention including amplification oligonucleotides, helper oligonucleotides and hybridization assay probes. In preferred embodiments, a kit of the present invention includes at least one amplification oligonucleotide and one hybridization assay probe capable of distinguishing Neisseria, Neisseria meningitidis or Neisseria gonorrhoeae from other microorganisms.

Brief Summary Text (34):
"Stringent" hybridization assay conditions refer to conditions wherein a specific hybridization assay probe is able to hybridize with target nucleic acids (preferably rRNA or rDNA of a Neisseria, Neisseria meningitidis or Neisseria gonorrhoeae) over other nucleic acids present in the test sample derived either from other microorganisms or from humans. It will be appreciated that these conditions may vary depending upon factors including the GC content and length of the probe, the hybridization temperature, the composition of the hybridization reagent or solution, and the degree of hybridization specificity sought. Specific stringent hybridization conditions are provided in the disclosure below.

Brief Summary Text (35):
As an example of specific stringent hybridization conditions useful in detecting Neisseria, Neisseria meningitidis or Neisseria gonorrhoeae, for the hybridization assay probes of this invention, a set of preferred stringent hybridization assay conditions was used. One preferred set comprised hybridizing the target nucleic acid and hybridization probe together in 100 .mu.l of 0.05 M lithium succinate (pH 5.0), 6 M LiCl, 1% (w/v) lithium lauryl sulfate, (L.L.S.) 10 mM ethylene diamine tetraacetic acid (EDTA), 10 mM ethylene glycol bis (beta-amino ethyl ether) N,N,N',N' tetraacetic acid (EGTA) at 60.degree. C. for 15 minutes, then adding 300 .mu.l of 0.15 M sodium tetraborate (pH 8.5), 1% (v/v) TRITON.RTM. X-100 at 60.degree. C. for 5-7 minutes. Additional sets of stringent hybridization conditions can be determined after reading the present disclosure by those or ordinary skill in the art.

Brief Summary Text (43):
By "conservatively modified variants" is meant nucleic acids or oligonucleotides having a nucleotide sequence that is complementary to a nucleic acid region of another nucleic acid, such region in turn being perfectly complementary to a reference nucleic acid. Such conservatively modified variants are able to stably hybridize to a target nucleic acid region having a Neisseria, Neisseria meningitidis or Neisseria gonorrhoeae nucleotide sequence under stringent hybridization conditions.

Brief Summary Text (55):
Probes specific for Neisseria meningitidis were designed using sequences determined in prospective target areas using primers complementary to the 16S rRNAs of strains of Neisseria including Neisseria gonorrhoeae (ATCC No. 19424), Neisseria meningitidis serogroup A (ATCC No. 13077), serogroup C (ATCC No. 23248) and serogroup L (ATCC No. 43828), clinical isolates of Neisseria meinigitidis, Neisseria lactamica (ATCC NO. 29193), Neisseria cinerea (ATCC NO. 14685), Neisseria mucosa (ATCC NO. 19696), Neisseria sicca (ATCC NO. 29193) and Kingella kingae (ATCC No. 23330). The nucleic acid sequence from phylogenetically near neighbors, including the published sequence of Neisseria gonorrhoeae NCTC 8375, Rossau et al. Nuc. Acids Res. 16:6227 were also used as comparisons with the nucleic sequences from Neisseria meningitidis to determine variable regions.

Brief Summary Text (57):
The sequence evolution at each of the variable regions is mostly divergent. Because of the divergence, more

distant phylogenetic relatives of Neisseria meningitidis or Neisseria gonorrhoeae tend to show greater variability in a given variable region than phylogenetically closer relatives. The observed sufficient variation between Neisseria meningitidis and Neisseria gonorrhoeae species was used to identify preferred target sites and design useful probes.

Brief Summary Text (58):
We have identified sequences which vary between Neisseria meningitidis and Neisseria gonorrhoeae, between these and other Neisseria species, and between members of the genus Neisseria and other organisms by comparative analysis of rRNA sequences published in the literature or determined in the laboratory. Computers and computer programs which may be used or adapted for the purposes herein disclosed are commercially available. We have seen sufficient variation between the target organisms and the closest phylogenetic relative likely to be found in the same sample to design the present probes. The Neisseria meningitidis strains have been classified into three sequence groups in the probe region represented by serogroups A, C and L.

Brief Summary Text (74):
Useful guidelines for designing amplification oligonucleotides and probes with desired characteristics are described herein. Our best mode target regions contain at least two and preferably three conserved regions of Neisseria meningitidis or Neisseria gonorrhoeae nucleic acid. These regions are about 15-350 in length; preferably 15-150 nucleotides in length.

Brief Summary Text (99):
D. Oligonucleotide Hybridization Assay Probes to Neisseria Meningitidis or Neisseria Gonorrhoeae rRNA and rDNA

Brief Summary Text (100):
The oligonucleotide hybridization assay probes disclosed and claimed herein are able to preferentially hybridize to target nucleic acids of Neisseria meningitidis rRNA or rDNA nucleotide sequences over nucleic acids of phylogenetically closely related bacterial species. These hybridization assay probes were designed, selected and/or chosen based upon a comparison of the nucleotide sequences of corresponding. regions of the ribosomal RNA of Neisseria meningitidis and said phylogenetically closely-related species. In preferred embodiments these probes selectively hybridize to the nucleic acids of Neisseria meningitidis over the nucleic acids of Neisseria gonorrhoeae.

Brief Summary Text (101):
The present invention contemplates oligonucleotide hybridization probes that selectively hybridize to the nucleic acids of Neisseria meningitidis and not to the nucleic acids of Neisseria gonnorhoeae and include Neisseria menigitidis nucleic acid sequences having or substantially corresponding to the following nucleic acid sequences: SEQ ID NO: 11 GGCTGTTGCT AATATCAGCG SEQ ID NO: 12 GGCTGTTGCT AATACCAGCG SEQ ID NO: 15 CGCTGATATT AGCAACAGCC SEQ ID NO: 16 CGCTGGTATT AGCAACAGCC SEQ ID NO: 25 GGCUGUUGCU AAUAUCAGCG SEQ ID NO: 26 GGCUGUUGCU AAUACCAGCG SEQ ID NO: 27 CGCUGAUAUU AGCAACAGCC SEQ ID NO: 28 CGCUGGUAUU AGCAACAGCC

Brief Summary Text (102):
A number of oligonucleotide hybridization assay probes of the present invention preferably hybridize to target nucleic acids containing Neisseria gonorrhoeae rRNA or rDNA nucleotide sequences over nucleic acids of other phylogenetically closely related bacterial species. In preferred embodiments, these hybridization assay probes can distinguish Neisseria gonorrhoeae nucleic acids from Neisseria meningitidis.

Brief Summary Text (103):

The hybridization probes of the present invention that selectively hybridize to nucleic acids derived from Neisseria gonorrhoeae and not to the nucleic acids of Neisseria meningitidis have or substantially correspond to the following nucleotide sequences: SEQ ID NO 1: *GAACGTACCG GGTAGCGG* SEQ ID NO 3: *GCCAATATCG GCGGCCGATG* SEQ ID NO 29: *CCGCTACCCG GTACGTTC* SEQ ID NO 30: *CATCGGCCGC CGATATTGGC* SEQ ID NO 31: *GAACGUACCG GGUAGCGG* SEQ ID NO 32: *GCCAAUAUCG GCGGCCGAUG* SEQ ID NO 33: *CCGCUACCCG GUACGUUC* SEQ ID NO 34: *CAUCGGCCGC CGAUAUUGGC*

Brief Summary Text (105):
The probe may also be combined with one or more unlabeled helper oligonucleotides to facilitate binding to the nucleic acid having the target Neisseria meningitidis or Neisseria gonorrhoeae nucleotide sequence. The probe then hybridizes to the target nucleic acid present in the sample; the resulting hybrid duplex may be separated. and detected by various techniques well known in the art, such as hydroxyapatite adsorption and radioactive monitoring. Also included among these techniques are those that involve selectively degrading the label present on unhybridized probe and then measuring the amount of label associated with the remaining hybridized probe, as disclosed in Arnold et al., U.S. Pat. No. 5,283,174, which enjoys common ownership with the present application and is incorporated by reference herein. This latter technique is presently preferred by the Applicants.

Brief Summary Text (109):
Specific helper oligonucleotides for facilitating the specific detection of Neisseria meningitidis nucleic acids have or substantially correspond to one of these nucleotide sequences: SEQ ID NO: 13 *GCCTTCGGGT TGTAAAGGAC TTTTGTCAGG GAAGAAAA* SEQ ID NO: 14 *GCTGATGACG GTACCTGAAG AATAAGCACC GGC* SEQ ID NO: 17 *TTTTCTTCCC TGACAAAAGT CCTTTACAAC CCGAAGGC* SEQ ID NO: 18 *GCCGGTGCTT ATTCTTCAGG TACCGTCATC AGC* SEQ ID NO: 35 *GCCUUCGGGU UGUAAAGGAC UUUUGUCAGG GAAGAAAA* SEQ ID NO: 36 *GCUGAUGACG GUACCUGAAG AAUAAGCACC GGC* SEQ ID NO: 37 *UUUUCUUCCC UGACAAAAGU CCUUUACAAC CCGAAGGC* SEQ ID NO: 38 *GCCGGUGCUU AUUCUUCAGG UACCGUCAUC AGC*

Brief Summary Text (110):
In preferred embodiments, hybridization probes directed to Neisseria meningitidis nucleic acids substantially correspond to SEQ ID NOS: 11, 12, 25 or 26 used in a probe mixture together with a helper oligonucleotide having or substantially corresponding to the nucleotide sequence of: SEQ ID NOS: 13, 14, 35 and 36

Brief Summary Text (111):
In other embodiments, a hybridization assay probe directed to Neisseria meningitidis nucleic acids substantially corresponding to SEQ ID NOS: 15, 16, 27 or 28

Brief Summary Text (122):
Compositions of the present invention include compositions for detecting Neisseria meningitidis nucleic acid comprising a nucleic acid hybrid formed between a nucleic acid derived from Neisseria meningitidis and an oligonucleotide having a nucleic acid sequence substantially corresponding to at least one of the nucleic acid sequences that follows: SEQ ID NO: 11 *GGCTGTTGCT AATATCAGCG* SEQ ID NO: 12 *GGCTGTTGCT AATACCAGCG* SEQ ID NO: 15 *CGCTGATATT AGCAACAGCC* SEQ ID NO: 16 *CGCTGGTATT AGCAACAGCC* SEQ ID NO: 25 *GGCUGUUGCU AAUAUCAGCG* SEQ ID NO: 26 *GGCUGUUGCU AAUACCAGCG* SEQ ID NO: 27 *CGCUGAUAUU AGCAACAGCC* SEQ ID NO: 28 *CGCUGGUAUU AGCAACAGCC* SEQ ID NO: 13 *GCCTTCGGGT TGTAAAGGAC TTTTGTCAGG GAAGAAAA* SEQ ID NO: 14 *GCTGATGACG GTACCTGAAG AATAAGCACC GGC* SEQ ID NO: 17 *TTTTCTTCCC TGACAAAAGT CCTTTACAAC CCGAAGGC* SEQ ID NO: 18 *GCCGGTGCTT ATTCTTCAGG TACCGTCATC AGC* SEQ ID NO: 35 *GCCUUCGGGU UGUAAAGGAC UUUGUCAGG GAAGAAAA* SEQ ID NO: 36 *GCUGAUGACG GUACCUGAAG AAUAAGCACC GGC* SEQ ID NO: 37 *UUUUCUUCCC UGACAAAAGU CCUUUACAAC CCGAAGGC* SEQ ID NO: 38 *GCCGGUGCUU AUUCUUCAGG UACCGUCAUC AGC* SEQ ID NO: 5 *GTCCCCTGCT TTCCCTCTCA AGAC* SEQ ID NO: 6 *GGCGAGTGGC GAACGGGTGA GTAACATA*

SEQ ID NO: 7 GCTGCTGCAC GTAGTTAGCC GGTGCTTATT CTTCAG SEQ ID NO: 8 GTTAGCCGGT GCTTATTCTT CAGGTACCGT CATCG SEQ ID NO: 9 CGGGTTGTAA AGGACTTTTG TCAGGGAAGA AAAGGCCGTT SEQ ID NO: 10GAAGGCCTTC GGGTTGTAAA GGAC SEQ ID NO: 41 GUCCCCUGCU UUCCCUCUCA AGAC SEQ ID NO: 42 GGCGAGUGGC GAACGGGUGA GUAACAUA SEQ ID NO: 43 GCUGCUGCAC GUAGUUAGCC GGUGCUUAUU CUUCAG SEQ ID NO: 44 GUUAGCCGGU GCUUAUUCUU CAGGUACCGU CAUCG SEQ ID NO: 45 CGGGUUGUAA AGGACUUUUG UCAGGGAAGA AAAGGCCGUU SEQ ID NO: 46 GAAGGCCUUC GGGUUGUAAA GGAC

Brief Summary Text (123):
Preferred compositions of the present invention include compositions for detecting Neisseria meningitidis comprising a nucleic acid hybrid formed between a nucleic acid derived from Neisseria meningitidis and an oligonucleotide having a nucleic acid sequence substantially corresponding to at least one of the nucleic acid sequences that follows: SEQ ID NO: 11 GGCTGTTGCT AATATCAGCG SEQ ID NO: 12 GGCTGTTGCT AATACCAGCG SEQ ID NO: 15 CGCTGATATT AGCAACAGCC SEQ ID NO: 16 CGCTGGTATT AGCAACAGCC SEQ ID NO: 25 GGCUGUUGCU AAUAUCAGCG SEQ ID NO: 26 GGCUGUUGCU AAUACCAGCG SEQ ID NO: 27 CGCUGAUAUU AGCAACAGCC SEQ ID NO: 28 CGCUGGUAUU AGCAACAGCC

Brief Summary Text (124):
The present invention also contemplates compositions for detecting Neisseria meningitidis having a nucleic acid hybrid formed between a Neisseria meningitidis-derived nucleic acid and a hybridization assay probe having a nucleic acid sequence substantially corresponding to: SEQ ID NO: 11 or SEQ ID NO: 25;

Brief Summary Text (126):
The present invention also contemplates compositions for detecting Neisseria meningitidis having a nucleic acid hybrid formed between a Neisseria meningitidis-derived nucleic acid and a hybridization assay probe having a nucleic acid sequence substantially corresponding to: SEQ ID NO: 12 or SEQ ID NO: 26;

Brief Summary Text (128):
The present invention also contemplates compositions for detecting Neisseria meningitidis having a nucleic acid hybrid formed between a Neisseria meningitidis-derived nucleic acid and a hybridization assay probe having a nucleic acid sequence substantially corresponding to: SEQ ID NO: 15 or SEQ ID NO: 27;

Brief Summary Text (130):
The present invention also contemplates compositions for detecting Neisseria meningitidis having a nucleic acid hybrid formed between a Neisseria meningitidis-derived nucleic acid and a hybridization assay probe having a nucleic acid sequence substantially corresponding to: SEQ ID NO: 16or SEQ ID NO: 28;

Brief Summary Text (136):
The present invention also contemplates compositions for detecting Neisseria meningitidis having a nucleic acid derived from Neisseria meningitidis and an oligonucleotide having a nucleic acid sequence substantially corresponding to SEQ ID NOS: 7 or 43

Brief Summary Text (138):
and optionally has a hybridization assay probe capable of hybridizing to a Neisseria meningitidis nucleic acid and which has a nucleic acid sequence substantially corresponding to one of the following nucleic acid sequences: SEQ ID NOS: 11, 15, 25 or 27

Brief Summary Text (140):
The present invention also contemplates compositions for detecting Neisseria meningitidis having a nucleic acid derived from Neisseria meningitidis and an oligonucleotide with a nucleic acid sequence substantially

corresponding to SEQ ID NOS: 7 or 43

Brief Summary Text (142):
and optionally has a hybridization assay probe capable of hybridizing to Neisseria meningitidis nucleic acids and which has a nucleic acid sequence substantially corresponding to one of the following nucleic acid sequences: SEQ ID NOS: 12, 16, 26 or 28

Brief Summary Text (144):
The present invention also contemplates compositions for detecting Neisseria meningitidis having a nucleic acid derived from Neisseria meningitidis and an oligonucleotide with a nucleic acid sequence substantially corresponding to SEQ ID NOS: 7 or 43

Brief Summary Text (146):
and optionally has a hybridization assay probe capable of hybridizing to a Neisseria meningitidis nucleic acid which has a nucleic acid sequence substantially corresponding to one of the following nucleic acid sequences: SEQ ID NOS: 15, 11, 27 or 25

Brief Summary Text (148):
The present invention also contemplates compositions for detecting Neisseria meningitidis having a nucleic acid derived from Neisseria meningitidis and an oligonucleotide with a nucleic acid sequence substantially corresponding to SEQ ID NOS: 7 or 43

Brief Summary Text (150):
and optionally has a hybridization assay probe capable of hybridizing to a Neisseria meningitidis nucleic acid which has a nucleic acid sequence substantially corresponding to one of the following nucleic acid sequences: SEQ ID NOS: 16, 12, 28 or 20

Brief Summary Text (152):
The present invention also contemplates compositions for detecting Neisseria meningitidis having a nucleic acid derived from Neisseria meningitidis and an oligonucleotide with a nucleic acid sequence substantially corresponding to SEQ ID NOS: 8 or 44

Brief Summary Text (154):
and optionally has a hybridization assay probe capable of hybridizing to a Neisseria meningitidis nucleic acid which has a nucleic acid sequence substantially corresponding to one of the following nucleic acid sequences: SEQ ID NOS: 15, 11, 27 or 25

Brief Summary Text (156):
The present invention also contemplates compositions for detecting Neisseria meningitidis having a nucleic acid derived from Neisseria meningitidis and an oligonucleotide with a nucleic acid sequence substantially corresponding to SEQ ID NOS: 8 or 44

Brief Summary Text (158):
and optionally has a hybridization assay probe capable of hybridizing to a Neisseria meningitidis nucleic acid which has a nucleic acid sequence substantially corresponding to one of the following nucleic acid sequences: SEQ ID NOS: 16, 12, 28 or 26

Brief Summary Text (177):
The present invention contemplates various methods for assaying for the presence of Neisseria meningitidis or Neisseria gonorrhoeae nucleic acid within a sample. One skilled in the art will understand that the exact assay

conditions, probes or primers used will vary depending on the particular assay format used and the source of the sample.

Brief Summary Text (178):
Generally, the present invention contemplates methods for detecting the presence of Neisseria meningitidis by contacting a test sample under stringent hybridization conditions with a nucleic acid hybridization assay probe capable of preferentially hybridizing under stringent hybridization assay conditions to a Neisseria meningitidis target nucleic acid over nucleic acids from Neisseria gonorrhoeae, said target nucleic acid having a nucleic acid sequence substantially corresponding to a sequence selected from the group consisting of: SEQ ID NO: 11 *GGCTGTTGCT AATATCAGCG* SEQ ID NO: 12 *GGCTGTTGCT AATACCAGCG* SEQ ID NO: 15 *CGCTGATATT AGCAACAGCC* SEQ ID NO: 16 *CGCTGGTATT AGCAACAGCC* SEQ ID NO: 25 *GGCUGUUGCU AAUAUCAGCG* SEQ ID NO: 26 *GGCUGUUGCU AAUACCAGCG* SEQ ID NO: 27 *CGCUGAUAUU AGCAACAGCC* SEQ ID NO: 28 *CGCUGGUAUU AGCAACAGCC*

Brief Summary Text (179):
Preferred methods for detecting the presence of Neisseria meningitidis include the step of contacting a test sample under stringent hybridization conditions with a nucleic acid hybridization assay probe capable of preferentially hybridizing under stringent hybridization assay conditions to a Neisseria meningitidis target nucleic acid sequence over nucleic acid sequences of Neisseria gonorrhoeae, said target nucleic acid sequence substantially corresponding to a sequence selected from the group consisting of: SEQ ID NO: 11 *GGCTGTTGCT AATATCAGCG* SEQ ID NO: 12 *GGCTGTTGCT AATACCAGCG* SEQ ID NO: 15 *CGCTGATATT AGCAACAGCC* SEQ ID NO: 16 *CGCTGGTATT AGCAACAGCC*

Brief Summary Text (180):
Preferred methods for detecting the presence of Neisseria gonorrhoeae include the step of contacting a test sample under stringent hybridization conditions with a nucleic acid hybridization assay probe capable of preferentially hybridizing under stringent hybridization assay conditions to aNeisseria gonorrhoeae target nucleic acid sequence over a nucleic acid sequence of Neisseria meningitidis, said target nucleic acid sequence substantially corresponding to a sequence selected from the group consisting of: SEQ ID NO 1: *GAACGTACCG GGTAGCGG* SEQ ID NO 3: *GCCAATATCG GCGGCCGATG* SEQ ID NO 29: *CCGCTACCCG GTACGTTC* SEQ ID NO 30: *CATCGGCCGC CGATATTGGC* SEQ ID NO 31: *GAACGUACCG GGUAGCGG* SEQ ID NO 32: *GCCAAUAUCG GCGGCCAUG* SEQ ID NO 33: *CCGCUACCCG GUACGUUC* SEQ ID NO 34: *CAUCGGCCGC CGAUAUUGGC*

Brief Summary Text (181):
In other embodiments, the present invention also contemplates methods for detecting the presence of Neisseria gonorrhoeae microorganisms by contacting a test sample under stringent hybridization conditions with a nucleic acid hybridization assay probe capable of preferentially hybridizing under stringent hybridization assay conditions to a Neisseria gonorrhoeae nucleic acid sequence over nucleic acid sequences from Neisseria meningitidis, said target nucleic acid sequences substantially corresponding to a sequence selected form the group consisting of: SEQ ID NO 1: *GAACGTACCG GGTAGCGG* SEQ ID NO 3: *GCCAATATCG GCGGCCGATG* SEQ ID NO 31: *GAACGUACCG GGUAGCGG* SEQ ID NO 32: *GCCAAUAUCG GCGGCCGAUG*

Brief Summary Text (185):
This first method step is then optionally followed by detecting the amplified nucleic acid produced in the amplification step with an oligonucleotide hybridization assay probe able to specifically hybridize to nucleic acids derived from Neisseria species, Neisseria cinerea, Neisseria meningitidis or Neisseria gonorrhoeae under stringent hybridization conditions.

Brief Summary Text (195):

The above methods may also include the further step of detecting the amplified nucleic acid with an oligonucleotide hybridization assay probe able to specifically hybridize to Neisseria meningitidis nucleic acids under stringent hybridization conditions.

Brief Summary Text (196):
Specifically, the methods may detect Neisseria meningitidis using oligonucleotide hybridization assay probes which will hybridize under stringent hybridization conditions to a nucleic acid sequence substantially corresponding to a sequence selected from the group consisting of: SEQ ID NO: 11 *GGCTGTTGCT AATATCAGCG* SEQ ID NO: 27 *CGCUGAUAUU AGCAACAGCC* SEQ ID NO: 12 *GGCTGTTGCT AATACCAGCG* SEQ ID NO: 28 *CGCUGGUAUU AGCAACAGCC* SEQ ID NO: 15 *CGCTGATATT AGCAACAGCC* SEQ ID NO: 25 *GGCUGUUGCU AAUAUCAGCG* SEQ ID NO: 16 *CGCTGGTATT AGCAACAGCC* SEQ ID NO: 26 *GGCUGUUGCU AAUACCAGCG*

Detailed Description Text (3):
Probes specific for Neisseria meningitidis were designed using sequences determined in prospective target areas using primers complementary to the 16S rRNAs of Neisseria gonorrhoeae (ATCC NO. 19424), Neisseria meningitidis sero group A (ATCC NOs 13077), serogroup C (ATCC No. 23248) and serogroup L (ATCC No. 43828), and clinical isolates, Neisseria lactamica (ATCC NO. 23970), Neisseria cinerea (ATCC NO. 14685), Neisseria mucosa (ATCC NO. 19696), Neisseria sicca (ATCC NO. 29193) and Kingella kingae (ATCC NO. 23330). The nucleic acid sequence from phylogenetically near neighbors, including the published sequence of Neisseria gonorrhoeae NCTC 8375 Rossau et al., Nuc. Acids Res. 16:6227 were also used as comparisons with the nucleic sequence from Neisseria meningitidis to determine variable regions.

Detailed Description Text (4):
An example of such an alignment follows: A specific sequence in which Neisseria meningitidis varied from E. coli and Neisseria gonorrhoeae was chosen for probe design. Two different probes were designed to Neisseria meningitidis (SEQ ID NO: 11) and (SEQ ID NO: 12). The rRNA sequences are shown below:

Detailed Description Text (17):
Sequence analysis of other Neisseria species indicated that the amplification oligonucleotides of this invention could amplify nucleic acids of other species. This example demonstrates the utility of the amplification oligonucleotides of this invention to amplify nucleic acid from another Neisseria species, N. meningitidis. In the course of development of a specific probe for N. meningitidis, it became clear that the members of the species N. meningitidis were not homogeneous in the probe region of choice. The sequences of 16S rRNAs of representative N. meningitidis species which showed low reactivity to the initial probe were determined and a second probe was designed. These data demonstrate the differential reactivity of three N. meningitidis species to the two probes. In this example, purified RNA from Neisseria gonorrhoeae (ATCC No. 19424), or lysates from Neisseria meningitidis serogroup A (ATCC No. 13077), serogroup C (ATCC No. 13102) and serogroup L, (ATCC No. 43828) representing approximately 1,000 cells were amplified with a promoter-primer and primer described in Example 5 under the conditions described in Example 5. Ten .mu.l samples of the 100 .mu.l amplification reactions were assayed by hybridization with an acridinium ester labeled probe synthesized with sequence 5'-*GCCAATATCGGCGGCCGATG*-3' (SEQ ID NO. 3 and an unlabeled helper probe synthesized with the sequence 5'-*ACGGTACCTGAAGAATAAGCACCGGCTAACTACGTG*-3, (SEQ ID NO. 4), or an acridinium ester labeled probe synthesized with the sequence 5'-*GGCTGTTGCTAATATCAGCG*-3' (SEQ ID NO. 11) and two unlabeled helper probes, one synthesized with sequence 5'-*GCCTTCGGGTTGTAAAGGACTTTTGTCAGGGAAGAAAA*-3' (SEQ ID NO. 13) and one synthesized with the sequence 5'-*GCTGATGACGGTACCTGAAGAATAAGCACCGGC*-3' (SEQ ID NO. 14), or an acridinium ester labeled probe synthesized with sequence 5'-*GGCTGTTGCTAATACCAGCG*-3' (SEQ ID NO. 12) with unlabeled helper probes SEQ ID NO: 13 and 14 or with a combination of labeled probes SEQ ID NO: 11 and 12 used with unlabeled helper probes SEQ ID NO: 13 and 14. Sequence analysis indicated that other strains of Neisseria

will also amplify with these primers.

Detailed Description Text (18):
The data show that strains of N. meningitidis and N. gonorrhoeae can be amplified using primers comprising SEQ ID NOs. 7 and 9 and detected with probes of SEQ ID NOs. 3, 11, and 12.

Detailed Description Text (20):
The sensitivity of the amplification and detection assay for N. meningitidis were demonstrated in this experiment. In this example, Neisseria meningitidis serogroup C cells were cultured and suspended in 0.9% sodium chloride to a density of approximately 10.sup.9 cells per ml. Cells were lysed following addition of an equal volume of a solution containing 3% (w/v) lithium lauryl sulfate, 30 mM sodium phosphate (pH 6.8), 1 mM EDTA, 1 mM EGTA and diluted with water prior to addition to the amplification reactions. Amplifications were performed as described for Example 5 using the promoter primer and primer described in Example 5 (SEQ ID NOs. 7 and 9, respectively). Twenty .mu.l of the reaction was analyzed by hybridization in the HPA format using an acridinium ester labeled probe synthesized with the sequence 5'-GGCTGTTGCTAATATCAGCG-3' (SEQ ID NO. 11) and two unlabeled helper probes, one synthesized with the sequence 5'-GCCTTCGGGTTG-TAAAGGACTTTTGTCAGGGAAGAAAA-3' (SEQ ID NO. 13) and one synthesized with the sequence 5'-GCTGATGACGGTACCTGAAGAATAAGCACCGGC-3' (SEQ ID NO. 14).

Detailed Description Text (22):
To demonstrate the reactivity and specificity of the probes directed to N. meningitidis 16S rRNA, a mixture of probes containing acridinium ester labeled oligonucleotides synthesized with the sequence 5'-CGCTGATATTAGCAACAGCC-3', (SEQ ID NO. 15) or sequence 5'-CGCTGGTATTAGCAACAGCC-3', (SEQ ID NO. 16), and unlabeled helper probes synthesized with the sequence 5'-TTTTCTTCCCTGACAAAAGTCCTTTACAACCCGAAGGC-3' (SEQ ID NO. 17 and 5'-GCCGGTGCTTATTCTTCAGGTACCGTCATCAG-3' (SEQ ID NO. 18), were hybridized to nucleic acid in lysates prepared from fresh cultures of the Neisseria species listed below. Each lysate was tested with a probe directed to a conserved region of 23S rRNA to confirm the lysis of the organism and integrity of the rRNA.

Detailed Description Text (23):
The data show that the mixture of probes allowed detection of all of the N. meningitidis strains tested. The probe mix did show a cross reaction with N. cinerea, an organism unlikely to be found in the same clinical samples as N. meningitidis. Treatment of patients with N. cinerea infections would be the same as for patients infected with N. meningitidis.

Detailed Description Text (26):
The data shown in the examples described above confirm that the novel amplification oligonucleotides herein described and claimed are capable of amplifying Neisseria nucleic acid and can be used in an assay to distinguish Neisseria meningitidis or Neisseria gonorrhoeae from each other, the closest known phylogenetic neighbours. None of the examples described herein are intended to limit the present invention to the embodiments of this disclosure, said invention being limited exclusively by the claims which follow.

Detailed Description Paragraph Table (1):
E. coli GAGUAAAG(UUAAUAC)CUUUG SEQ ID NO: 54 . . .. GGCTGTTG(CTAATAC)CAGCG SEQ ID NO: 12 .... GGCTGTTG(CTAATAT)CAGCG SEQ ID NO: 11 ... . N. meningitidis.c GGCUGUUG(CUAAUAU)CAGCG SEQ ID NO: 55 .... N. gonorrhoeae.P GGCUGUUG(CCAAUAU)CGGGG SEQ ID NO: 56

Detailed Description Paragraph Table (7):

TABLE 6 Amplification of Neisseria gonorrhoeae and Neisseria meningitidis strains using primers comprising SEQ ID NOs. 7 and 9. RLU Probe SEQ ID NOs: 3 11 12 11 + 12 Helper probe 4 13 + 14 13 + 14 13 + 14 SEQ ID NOs: Organism N. gonorrhoeae 1,017,626 1,660 820 1,603 994,788 1,448 809 1,559 1,030,242 1,743 805 1,792 N. meningitidis 2,059 1,208,967 3,534 829,251 Serogroup A 1,861 1,115,956 3,700 760,360 2,183 1,138,675 3,546 775,675 N. meningitidis 1,931 1,164,254 2,819 749,502 Serogroup C 2,130 1,068,489 2,477 687,517 1,963 1,110,933 3,103 803,732 N. meningitidis 1,833 85,321 1,206,045 1,537,314 Serogroup L 1,972 79,555 1,199,815 1,474,016 1,814 77,797 1,211,022 1,645,742

Detailed Description Paragraph Table (8):
TABLE 7 Amplification of N. meningitidis serogroup A with amplification oligomers comprising SEQ ID NOs. 7 and 9, followed by detection with probe SEQ ID NO. 11. RLU with Amount of probe SEQ ID target added NO. 11 40 cells 723,645 648,069 686,492 4 cells 195,370 189,451 162,128 0.4 cells 28,585 23,253 824,742 64,945 0 cells 1,432 1,202 1,258

Detailed Description Paragraph Table (9):
TABLE 8 Reactivity and specificity of probes directed to N. meningitidis 16S rRNA. RLU with ATCC RLU with conserved Organism No. probe mix* probe N. cinerea 14685 736,927 59,831 N. denitrificans 14686 581 50,391 N. elongata 25295 1,511 52,017 N. elongata subspe- 29315 618 53,312 cies glycolytica N. flavescens 13120 1,316 53,397 N. gonorrhoeae 9793 1,826 62,658 N. gonorrhoeae 9827 753 60,252 N. gonorrhoeae 9830 4,832 58,346 N. gonorrhoeae 10150 1,139 61,573 N. gonorrhoeae 10874 759 58,291 N. gonorrhoeae 11689 4,824 60,039 N. gonorrhoeae 19088 910 53,594 N. gonorrhoeae 19424 851 60,372 N. gonorrhoeae 21824 746 62,153 N. gonorrhoeae 27630 1,829 53,241 N. gonorrhoeae 33084 784 62,696 N. gonorrhoeae 35541 431 59,229 N. lactamica 23970 3,497 54,255 N. meningitidis ser- 13077 844,739 54,292 ogroup A N. meningitidis ser- 23255 722,108 61,439 ogroup B N. meningitidis ser- 13090 704,890 57,321 ogroup B N. meningitidis ser- 23251 761,475 58,545 ogroup B N. meningitidis ser- 13103 770,221 63,704 ogroup C N. meningitidis ser- 13106 761,099 60,928 ogroup C N. meningitidis ser- 13102 752,743 62,351 ogroup C N. meningitidis ser- 13111 711,196 59,635 ogroup C N. meningitidis ser- 13109 768,874 63,295 ogroup C N. meningitidis ser- 13110 676,060 58,150 ogroup C N. meningitidis ser- 13112 543,492 54,921 ogroup C N. meningitidis ser- 23248 321,600 59,308 ogroup C N. meningitidis ser- 13113 770,893 56,429 ogroup D N. meningitidis 35558 797,072 58,882 group E N. meningitidis ser- 43828 559,406 61,534 ogroup L N. meningitidis ser- 43744 705,798 62,152 ogroup W-135 N. meningitidis ser- 35561 778,600 54,938 ogroup Y N. meningitidis ser- 35562 749,756 61,793 ogroup Z N. meningitidis 13095 726,612 52,614 N. meningitidis 13101 775,912 59,839 N. meningitidis 13804 785,737 61,790 N. meningitidis 43743 734,400 61,357 N. mucosa 19696 1,560 53,427 N. mucosa subspecies 25999 1,761 59,306 heidelbergensis N. sicca 29193 1,205 58,260 N. sicca 9913 2,203 57,764 N. subflava 14799 2,046 50,832 Negative sample 5,251 124 467 132 1,691 138
*probe mix contained acridinium ester labeled probes synthesized with sequences of SEQ ID NO. 15 and SEQ ID NO. 16 and unlabeled helper probes synthesized with sequences of SEQ ID NO. 17 and SEQ.ID NO. 18.

Detailed Description Paragraph Table (10):
TABLE 9 Specificity of an assay using amplification with oligonucleotides comprising SEQ ID NOs. 7 and 9 followed by detection with probes comprising SEQ ID NOs. 3, 11 or 12. RLU SEQ ID NOs.: Probe Probe to 11 12 3 conserved regions of bacterial rRNA Helpers 13 + 14 13 + 14 4 Organism ATCC No. Neisseria 14685 2,468,721 540,699 1,804 1,633 cinerea 609,648 2,484 1,536 575,050 1,943 1,494 Neisseria 14686 2,339,034 740 644 1,563 denitrificans 659 578 1,539 Neisseria 25295 2,486,745 772 428 1,521 elongata 738 3,297 1,528 Neisseria 29315 2,397,697 697 431 1,443 elongata 954 813 1,528 subspecies glycolytica Neisseria 13120 2,622,452 780 493 1,547 flavescens 874 481 1,610 969 429 1,589 Neisseria 23970 2,299,619 736 410 1,621 lactamica 839 425 1,544 1,583 428 1,559 Neisseria 19696 2,565,699 1,021 981 1,596 mucosa 1,408 559 6,781 851 5,260 1,574 Neisseria 25999 2,927,147 653 367 1,430 mucosa 664 390 1,971 heidelber- gensis Neisseria 9913 2,427,561 699 777 1,609 sicca 847 477 1,552 834 437 1,642 Neisseria 29193 2,804,642 954 423 1,588 sicca 615 388 1,505 Neisseria 19424 N.T. 3,826 419 586,358 gonorrhoeae* 1,092 411 564,987

2,390 388 554,134 Neisseria 13077 N.T. 557,656 1,287 1,492 meningitidis* 621,180 1,009 1,509 Sero-539,592 954 1,617 group A *purified RNA used at 500 pg per reaction. N.T. = Not tested.

Other Reference Publication (2):
Auriol et al., "Characterization of serogroup A Neisseria meningitidis strains by rRNA gene restriction patterns and PCR: correlation with results of serotyping, subtyping and multilocus enzyme electrophoresis," FEMS Immunology and Medical Microbiology 10:219-226 (1995).

Other Reference Publication (9):
McLaughlin et al., "Amplification of rDNA loci to detect and type Neisseria meningitidis and other eubacteria," Molecular and Cellular Probes 7:7-17 (1993).

Other Reference Publication (13):
Radstrom et al., "Detection of bacterial DNA in cerbrospinal fluid by an assay for simultaneous detection of Neisseria meningitidis, Haemophilus influenzae, and Streptococci using a seminested PCR strategy," J. Clin. Microbiol. 32(11):2738-2744 (1994).

Other Reference Publication (18):
Wolff et al., "Phylogeny and nucleotide sequence of a 23S rRNA gene from Neisseria gonorrhoeae and Neisseria meningitidis," Nucleic Acids Research 20(17):4657 (1992).

CLAIMS:

1. A hybridization assay probe for use in detecting the presence of Neisseria meningitidis in a sample, wherein the base sequence of said probe consists of the base sequence of a first sequence selected from the group consisting of: SEQ ID NO. 11; SEQ ID NO. 15; SEQ ID NO. 25; and SEQ ID NO. 27.

2. The probe of claim 1, wherein said first sequence is SEQ ID NO. 11.

3. The probe of claim 1, wherein said first sequence is SEQ ID NO. 15.

4. The probe of claim 1, wherein said first sequence is SEQ ID NO. 25.

5. The probe of claim 1, wherein said first sequence is SEQ ID NO. 27.

6. A kit comprising: a hybridization assay probe for use in detecting the presence of Neisseria meningitidis in a sample, wherein the base sequence of said probe consists of the base sequence of a first sequence selected from the group consisting of: SEQ ID NO. 11; SEQ ID NO. 15; SEQ ID NO. 25; and SEQ ID NO. 27; and at least one helper probe, wherein the base sequence of said helper probe consists of the base sequence of a second sequence selected from the group consisting of: SEQ ID NO. 13; SEQ ID NO. 14; SEQ ID NO. 17; SEQ ID NO. 18; SEQ ID NO. 35; SEQ ID NO. 36; SEQ ID NO. 37; and SEQ ID NO. 38.

7. The kit of claim 6, wherein: said first sequence is selected from the group consisting of: SEQ ID NO. 11; and SEQ ID NO. 25; and said second sequence is selected from the group consisting of: SEQ ID NO. 13; SEQ ID NO. 14; SEQ ID NO. 35; and SEQ ID NO. 36.

8. The kit of claim 6, wherein: said first sequence is selected from the group consisting of: SEQ ID NO. 15; and SEQ ID NO. 27; and said second sequence is selected from the group consisting of: SEQ ID NO. 17; SEQ ID NO. 18; SEQ ID NO. 37; and SEQ ID NO. 38.

9. The kit of claim 6, wherein said at least one helper probe includes first and second helper probes, wherein: said first sequence is SEQ ID NO. 11; said second sequence is SEQ ID NO. 13 for said first helper probe; and said second sequence is SEQ ID NO. 14 for said second helper probe.

10. The kit of claim 6, wherein said at least one helper probe includes first and second helper probes, wherein: said first sequence is SEQ ID NO. 15; said second sequence is SEQ ID NO. 17 for said first helper probe; and said second sequence is SEQ ID NO. 18 for said second helper probe.

11. A kit comprising: a hybridization assay probe for use in detecting the presence of Neisseria meningitidis in a sample, wherein the base sequence of said probe consists of the base sequence of a first sequence selected from the group consisting of: SEQ ID NO. 11; SEQ ID NO. 15; SEQ ID NO. 25; and SEQ ID NO. 27; and at least one amplification oligonucleotide, wherein the base sequence of said amplification oligonucleotide consists of the base sequence of a second sequence selected from the group consisting of: SEQ ID NO. 7; SEQ ID NO. 9; SEQ ID NO. 43; and SEQ ID NO. 45, or

wherein the base sequence of said amplification oligonucleotide consists of a 3' base sequence which is perfectly homologous to said second sequence and a 5' base sequence which is recognized by an RNA polymerase or which enhances initiation or elongation by an RNA polymerase.

12. The kit of claim 11, wherein the base sequence of said amplification oligonucleotide consists of the base sequence of said second sequence.

13. The kit of claim 11, wherein said second sequence is SEQ ID NO. 7 or SEQ ID NO. 43.

14. The kit of claim 11, wherein said second sequence is SEQ ID NO. 9 or SEQ ID NO. 45.

15. The kit of claim 11, wherein said at least one amplification oligonucleotide includes first and second amplification oligonucleotides, wherein: said second sequence is SEQ ID NO. 7 or SEQ ID NO. 43 for said first amplification oligonucleotide; and said second sequence is SEQ ID NO. 9 or SEQ ID NO. 45 for said second amplification oligonucleotide.

16. The kit of claim 11, wherein said first sequence is SEQ ID NO. 11.

18. A composition comprising a nucleic acid hybrid formed between said probe and said first target region of any one of claims 2-5.

19. A probe mix comprising the probe of claim 1 and at least one helper probe, wherein the base sequence of said helper probe consists of the base sequence of a second sequence selected from the group consisting of: SEQ ID NO. 13; SEQ ID NO. 14; SEQ ID NO. 17; SEQ ID NO. 18; SEQ ID NO. 35; SEQ ID NO. 36; SEQ ID NO. 37; and SEQ ID NO. 38.

20. The probe mix of claim 19, wherein: said first sequence is selected from the group consisting of: SEQ ID NO. 11; and SEQ ID NO. 25; and said second sequence is selected from the group consisting of: SEQ ID NO. 13; SEQ ID NO. 14; SEQ ID NO. 35; and SEQ ID NO. 36.

21. The probe mix of claim 19, wherein: said first sequence is selected from the group consisting of: SEQ ID NO. 15; and SEQ ID NO. 27; and said second sequence is selected from the group consisting of: SEQ ID NO. 17; SEQ ID NO. 18; SEQ ID NO. 37; and SEQ ID NO. 38.

22. The probe mix of claim 19, wherein said at least one helper probe includes first and second helper probes,

wherein: said first sequence is SEQ ID NO. 11; said second sequence is SEQ ID NO. 13 for said first helper probe; and said second sequence is SEQ ID NO. 14 for said second helper probe.

23. The probe mix of claim 19, wherein said at least one helper probe includes first and second helper probes, wherein: said first sequence is SEQ ID NO. 15; said second sequence is SEQ ID NO. 17 for said first helper probe; and said second sequence is SEQ ID NO. 18 for said second helper probe.

24. A method for detecting the presence of Neisseria meningitidis subtypes A, C and L in a sample, said method comprising the steps of: (a) contacting said sample with said probe of claim 1 or 17 under stringent hybridization assay conditions; and (b) detecting the presence of said probe as an indication of the presence of at least one of Neisseria meningtidis subtypes A, C and L in said sample.

25. The method of claim 24, wherein said first sequence is SEQ ID NO. 11.

26. The method of claim 24, wherein said first sequence is SEQ ID NO. 15.

27. The method of claim 24, wherein said first sequence is SEQ ID NO. 25.

28. The method of claim 24, wherein said first sequence is

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Importance: High

Please search, oligomer search, probe, primer and homolog search SEQ ID NO 95. (nucleic acid claims.)

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95 - 284 NA

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Searcher:_____
Phone:_____
Location:_____
Date Picked Up: 11/5/03
Date Completed: 11/6/03
Searcher Prep/Review:_____
Clerical:_____
Online time:_____

TYPE OF SEARCH:
NA Sequences: 1
AA Sequences:_____
Structures:_____
Bibliographic:_____
Litigation:_____
Full text:_____
Patent Family:_____
Other:_____

VENDOR/COST (where applic.)
STN:_____
DIALOG:_____
Questel/Orbit:_____
DRLink:_____
Lexis/Nexis:_____
Sequence Sys.: 03H
WWW/Internet:_____
Other (specify):_____